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Hepatitis E Virus Immunoglobulin G Antibodies in Different Populations in Campinas, Brazil

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The seroprevalence of anti-hepatitis E virus (HEV) antibodies was investigated by enzyme immunoassay in 205 volunteer blood donors, 214 women who attended a center for anonymous testing for human immunodeficiency virus (HIV) infection, and 170 hospital employees in Campinas, a city in southeastern Brazil. The prevalence of anti-HEV antibodies ranged from 2.6% (3 of 117) in health care professionals to 17.7% (38 of 214) in women who considered themselves at risk for HIV. The prevalence of anti-HEV antibodies in health care professionals was not significantly different from that in healthy blood donors (3.0%, 5 of 165) and blood donors with raised alanine aminotransferase levels (7.5%, 3 of 40). The prevalence of anti-HEV antibodies (13.2%, 7 of 53) in cleaning service workers at a University hospital was similar to that among women at risk for HIV infection. These results suggest that HEV is circulating in southeastern Brazil and that low socioeconomic status is an important risk factor for HEV infection in this region.

Hepatitis E virus (HEV) is considered the main etiologic agent of enterically transmitted non-A, non-B hepatitis (ET-NANBH) and occurs in epidemics or sporadically. ET-NANBH, once thought to be a disease confined to developing countries, is now recognized to have a wider geographical distribution (12, 33). Epidemics have been related to contaminated water supplies, as fecal-oral transmission is the major route of transmission (29). The symptoms of ET-NANBH are similar to those of hepatitis A, although it affects primarily young adult populations already immune to hepatitis A virus (HAV). HEV is well recognized as a cause of fulminant hepatic failure in areas where it is endemic (23), particularly in pregnant women who contract it in the third trimester (10). In developed countries, sporadic cases have been identified among travelers from areas where it is endemic and HEV has been implicated in some community-acquired cases of NANBH in the United States and other western countries (12).

Until recently, the diagnosis of ET-NANBH was based on serology after the exclusion of other viral hepatitis. In 1990, the isolation of a partial cDNA clone from HEV (22) led to the identification of type-common immunodominant epitopes and the development of diagnostic serological assays for the detection of antibodies to recombinant HEV antigens (4).

The prevalence of HEV infection among blood donors in developed countries ranges from 0.4 to 3.9% (4, 14, 15). An association between HEV and hepatitis C virus infections has been reported, suggesting similar or overlapping routes of transmission (21). In addition, a higher prevalence of antibodies to HEV has been reported among patients undergoing chronic hemodialysis (9), suggesting that this virus is also spread by the parenteral route. Homosexual men also have a high prevalence of HEV infection (15), and the possibility of sexual transmission cannot be neglected.

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Few studies have addressed the prevalence of HEV infection in Brazil because diagnostic tests for this illness have only recently been available. HEV infection has already been detected in the Amazon basin among gold miners (17) and isolated communities (26). Acute viral hepatitis cases possibly associated with HEV have been reported in central (27) and northeastern Brazil (18, 19). In southeastern Brazil, HEV antibodies have been detected in health care workers and dialysis patients (6).

The aim of the present study was to determine the prevalence of HEV infection among blood donors and populations with different risks of exposure to viral infections, such as women attending a center for anonymous testing for human immunodeficiency virus (HIV) infection (CAT-HIV) and employees working at the State University of Campinas hospital, Campinas, São Paulo State, in southeastern Brazil.

MATERIALS AND METHODS

Population. A total of 589 samples collected in Campinas, São Paulo State, Brazil, were analyzed. The samples were from individuals in three different groups.

Group I. One hundred sixty-five volunteer blood donors with alanine aminotransferase (ALT) levels <2 times the upper normal value (129 [78.2%] male and 36 [21.8%] female; mean age, 33.9 ± 10.1 years; range, 18 to 61 years; median, 32 years [group IA]) and 40 volunteer blood donors with ALT levels >2 times the upper normal value (39 [90%] male and 4 [10%] female; mean age, 34.3 ± 7.9 years; range, 21 to 54 years; median, 34 years [group IB]) were included. These 205 donors were all negative for the routinely screened markers of syphilis, hepatitis B and C, HIV type 1 and 2 infections, human T-cell leukemia virus type 1 and 2 infections, and Chagas' disease.

Group II. Two hundred fourteen women (mean age, 29.6 ± 10.2 years; range, 14 to 71 years; median, 26 years) who attended a CAT-HIV were included in group II, which was further divided into groups IIC (21 prostitutes) and IID (193 women who denied prostitution but considered themselves at risk for HIV infection). Data on several variables (intravenous drug use [IVDU], previous sexually transmitted disease [STD], practice of oral or anal sex, and anti-hepatitis B core antigen and anti-hepatitis C virus [HCV] antibody status) possibly related to a risk of viral transmission were collected from this population.

Group III. One hundred seventy employees working in the State University of Campinas hospital (35 [20.5%] male and 135 [71.5%] female; mean age, 33.2 ± 8.2 years; range, 20 to 53 years; median, 31 years). This group was further divided into 117 health care workers (group IIIE) and 53 cleaning service workers (group IIIF).

TABLE 1. Seroprevalence of anti-HEV IgG antibodies in different populations

Group	No. (%) of persons		Total	*P* value
	Anti-HEV antibody positive	Anti-HEV antibody negative		
I	8 (4.0)	197 (96.0)	205	0.1889
IA	5 (3.0)	160 (97.0)	165	
IB	3 (7.5)	37 (92.5)	40	
II	38 (17.7)	176 (82.3)	214	1.0000
IIC	3 (14.2)	18 (85.8)	21	
IID	35 (18.1)	158 (81.9)	193	
III	10 (5.9)	160 (94.1)	170	0.0129
IIIE	3 (2.6)	114 (97.4)	117	
IIIF	7 (13.2)	48 (86.8)	53	

Serology. Serum samples were stored at −20°C until tested for anti-HEV immunoglobulin G (IgG) using an enzyme immunoassay (Abbott GmbH Diagnostika, Wiesbaden, Germany). This assay is based on two recombinant antigens (SG-3 and 8-5) derived from different open reading frames of the Burmese strain of HEV expressed as a CMP-2-keto-3-deoxyoctulosonic acid synthetase fusion protein in *Escherichia coli*. Samples were tested in accordance with the manufacturer's instructions, and those with absorbances less than the cutoff (CO) value were considered negative. Samples with absorbance (S) greater than or equal to the CO value were tentatively considered reactive and then retested in duplicate to confirm the result. Results were recorded as S/CO ratios to allow comparison of the intensity reactions of individual samples. The samples were considered reactive when the S/CO ratio was higher than 1.0.

Antibodies to HCV were detected using a third-generation enzyme immunoassay (Abbott Laboratories, North Chicago, Ill.). Anti-HBc antibodies were screened with a competitive enzyme immunoassay (Corzyme; Abbott Laboratories).

Statistical analysis. Statistical comparison of distribution was carried out using either the χ^2 or Fisher exact test, as applicable. For comparison of means and variances, other tests were used (Student *t*, Kruskal-Wallis, and Mann-Whitney tests). The level of significance was set at $P < 0.05$.

RESULTS

As shown in Table 1, the prevalence of anti-HEV IgG antibodies was 4.0, 17.7, and 5.9% in groups I, II, and III, respectively. The prevalence of anti-HEV IgG antibodies in group II was significantly higher ($P < 0.001$) than in groups I and III.

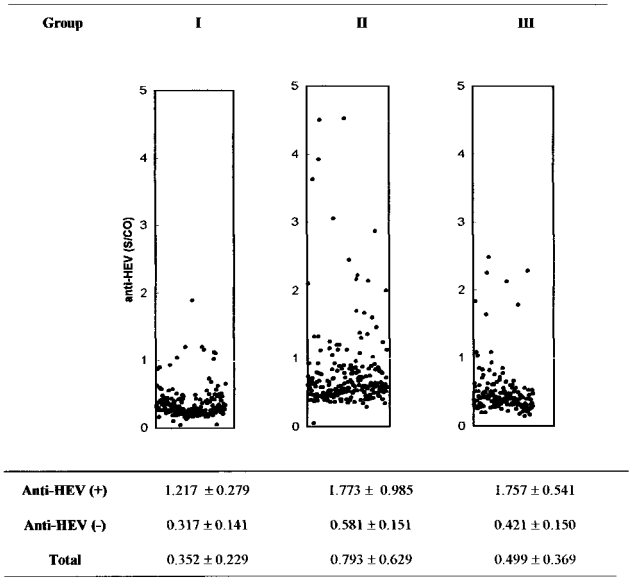
Anti-HEV IgG antibodies were found in 5 of 165 (3.0%) and in 3 of 40 (7.5%) blood donors with ALT levels <2 times (group IA) and >2 times (group IB) the upper normal value, respectively. The two groups had similar prevalences of anti-HEV antibodies ($P = 0.1889$).

Anti-HEV IgG was detected in 3 (14.2%) out of 21 prostitutes and in 35 (18.1%) out of 193 women who denied prostitution in group II. There was no statistically significant difference in the prevalence of anti-HEV between these two populations ($P = 1.000$).

In group III, the prevalence of anti-HEV IgG was significantly different between the two subgroups: 3 of 117 (2.6%) among health care workers but 7 of 53 (13.2%) among cleaning service workers ($P = 0.0129$).

The anti-HEV reactivity of sera from these populations was also analyzed (Fig. 1). For blood donors, the mean S/CO ratio was 0.352 ± 0.229 (range, 0.040 to 1.890). For group II, the mean S/CO ratio was 0.793 ± 0.629 (range, 0.050 to 4.520). For group III, the mean S/CO ratio was 0.499 ± 0.369 (range, 0.150 to 2.480). The mean S/CO ratios were different among these three groups ($P < 0.001$).

Considering only the anti-HEV antibody-positive samples, the mean S/CO ratio of samples from blood donors (1.217 ± 0.279) was significantly different from those found in groups II (1.773 ± 0.985; $P = 0.005$) and III (1.757 ± 0.541; $P = 0.016$). The mean S/CO ratios of positive samples from groups II and III were not significantly different ($P = 0.947$). Among the negative samples, the mean S/CO ratios were significantly different among the three groups (group I, 0.317 ± 0.141; group II, 0.581 ± 0.151; group III, 0.421 ± 0.150; $P < 0.001$).



	I	II	III
Anti-HEV (+)	1.217 ± 0.279	1.773 ± 0.985	1.757 ± 0.541
Anti-HEV (-)	0.317 ± 0.141	0.581 ± 0.151	0.421 ± 0.150
Total	0.352 ± 0.229	0.793 ± 0.629	0.499 ± 0.369

FIG. 1. Anti-HEV reactivity of sera from the different groups. Values are expressed as S/CO ratios. Mean S/CO ratios ± standard deviations are shown in the bottom.

The variables possibly related to viral transmission among women who attended the CAT-HIV are shown in Table 2. The prevalence of anti-HEV antibodies was not significantly related to IVDU, previous STD, or practice of oral or anal sex. Anti-HBc antibodies were detected in 36 of 214 (16.8%) women tested, while anti-HCV antibodies were detected in 10 of 214 (4.7%) women tested. There was no significant association between the detection of anti-HEV antibodies and serological markers of hepatitis B and C.

DISCUSSION

The overall prevalence of anti-HEV antibodies among blood donors from Campinas (4%) was generally higher than that reported in studies from developed countries (0.4 to 3.9%) (4, 13, 14). The anti-HEV antibody prevalence found in blood donors from Campinas was similar to that reported for blood donors from Salvador, Bahia, northeastern Brazil (2%; $P = 0.2590$) (18). On the other hand, it was lower than that in countries such as China, where a prevalence of 16% ($P < 0.001$) was reported (13). Furthermore, recently published data from another South American population, Bolivian blood donors, showed a prevalence of 16.2% (93 of 574; $P = 0.00001$) (11). The large difference found in anti-HEV antibody prevalence reinforces the importance of studies involving other populations from different geographical regions.

The prevalence of anti-HEV antibodies did not differ between blood donors with normal and raised ALT levels, suggesting that HEV is not a common cause of raised ALT levels in patients without markers of known viruses. Our results agree with previous data showing that viral hepatitis is not a common

TABLE 2. Variables possibly related to HEV transmission among women attending a CAT-HIV

Variable	Presence of variable	No. (%) of persons		Total	*P* value
		Anti-HEV antibody positive	Anti-HEV antibody negative		
IVDU	Yes	0 (0)	5 (100.0)	5	0.5886
	No	38 (18.2)	171 (81.8)	209	
STD	Yes	4 (12.5)	28 (87.5)	32	0.6153
	No	34 (18.7)	148 (81.3)	182	
Oral sex	Yes	22 (18.6)	96 (81.4)	119	0.7066
	No	16 (16.7)	80 (83.4)	96	
Anal sex	Yes	16 (23.5)	52 (76.5)	68	0.1315
	No	22 (16.7)	124 (84.9)	146	
Anti-HBc antibodies	Yes	9 (25.0)	27 (75.0)	36	0.2124
	No	29 (16.3)	149 (83.7)	178	
Anti-HCV antibodies	Yes	3 (30.0)	7 (70.0)	10	0.3880
	No	35 (17.2)	169 (82.8)	204	

cause of raised ALT levels in seronegative blood donors (8). Only an IgM-specific assay can distinguish between acute and past HEV infections (32), but it is noteworthy that anti-HEV antibody-positive blood donors have a significantly lower mean S/CO ratio (1.217 ± 0.279) than the other groups. These positive IgG results probably reflect previous but not acute exposure to the virus.

The prevalence of anti-HEV antibodies in blood donors from Campinas was lower than that of anti-HAV antibodies (20). This fact can be explained four different ways.

First, transmission of HEV seems to be less efficient than that of HAV; as HEV appears to be extremely labile (1, 2) while a major distinguishing feature of HAV is its exceptional stability (24).

Second, the kinetics of anti-HEV antibody decay may be different and some individuals previously infected may not be detected. Controversial data have already been published: two-thirds of pediatric cases became seronegative by 9 months in one study, but another study showed positive cases in adults infected 14 years before (3).

Third, there are no data on the time of introduction of HEV into the local population. We may speculate that HEV was introduced more recently in southeastern Brazil. Studies comparing viral RNA sequences from Brazilian isolates by phylogenetic analysis may be useful in addressing this point.

Fourth, currently commercial available kits for the detection of anti-HEV IgG may not be sensitive enough to detect antibodies directed to different epitopes expressed in some viral proteins or only by some viral strains.

Further studies using recently developed anti-HEV kits and involving follow-up of anti-HEV antibody-positive individuals and the molecular characterization of circulating HEV genotypes should be carried out to elucidate the role of these hypotheses to explain these results.

The first anti-HEV kits were based on immunoreactive regions located at open reading frames 2 and 3 (30). The assay commercially available in our country also has peptide SG3 as an antigen, leading to increased sensitivity since it contains two additional epitopes (31). This assay has already been used worldwide for epidemiological studies, but improved assays for anti-HEV detection are still under development.

Recently, the accuracy of these assays has been improved using other antigens, such as a mosaic protein containing short antigenically reactive regions of the virus (5) or recombinant proteins that have a conformational and immunological structure similar to that of native antigens (34). This last assay has been shown to have higher sensitivity than the ones developed first, especially concerning the detection of IgM antibodies (32).

Only 3 (2.6%) out of 117 health care workers (doctors, nurses, and laboratory staff) had anti-HEV antibodies, showing that they do not constitute a risk group for HEV infection. On the other hand, a statistically significantly higher prevalence (7 of 53 [13.2%]; $P = 0.0129$) of anti-HEV antibodies was found among cleaning service workers.

A statistically significantly higher prevalence of anti-HEV antibodies was also found in women from group II (17.7%). There was no difference in anti-HEV antibody frequency between the two subgroups (C and D) of these women. The prevalence of anti-HBc (16.8%) and anti-HCV (4.7%) antibodies among these women was higher than in blood donors (9 and 2.1%, respectively; $P < 0.05$) (7). This could reflect either their socioeconomic status or their lifestyle, since exposure to sexual or parenteral transmission is a known risk factor for hepatitis B or C infection, respectively.

Anti-HEV antibodies have not been detected in a significantly higher proportion in women positive for anti-HBc and anti-HCV antibodies, which are surrogate markers of sexual and parenteral risks of exposure, respectively. Furthermore, the anti-HEV antibody results for these women showed no significant correlation with risk factors previously associated with HEV infection by other authors, such as IVDU (28) or sexual promiscuity (16), measured by the number of partners and previous STD. Sexual practices such as oral and anal sex, which are potentially associated with transmission by the fecal-oral route, were not associated with anti-HEV antibodies.

The overall prevalence among the cleaning service workers (13.2%) in group IIIF was similar to that observed in group II ($P = 0.4283$), indicating that both populations have an increased risk for HEV infection.

Groups II and III showed the highest mean S/CO ratio compared to group I, suggesting that these groups probably have a more recent contact with HEV, either as the first infection or as an immunological booster. Only the specific de-

tection of anti-HEV IgM antibodies could distinguish recent from past HEV infections. No statistically significant difference in the mean S/CO ratios was found between these two groups, although samples with ratios higher than 3.00 were found only in group II.

The commercially available enzyme-linked immunosorbent assay kit detects specific anti-HEV IgG and therefore is useful in diagnosing acute and past HEV infections. The recent development of an HEV IgM enzyme-linked immunosorbent assay was necessary to confirm acute HEV infection. Recently, two new assays have been developed using either a mosaic protein (5) or baculovirus-expressed antigens (32) but neither of them is commercially available in our country.

Two explanations could account for the higher exposure to HEV among groups II and IIIF compared to the other groups. The more probable explanation is their socioeconomic status, as cleaning service workers are generally recruited in poor, peripheral districts from populations with a low socioeconomic status, as well as most of the women who attended the CAT-HIV. Another explanation is directly related to their lifestyle. The activities of cleaning service workers make them more prone to exposure to agents transmitted by the fecal-oral route. Among the women who attended the CAT-HIV, we cannot rule out other factors related to their behavior not analyzed that might have contributed to the higher exposure to HEV. The data presented here points to socioeconomic status as the more likely hypothesis, but studies involving individuals of low socioeconomic status not exposed to the same conditions as groups II and IIIF are required to confirm it.

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